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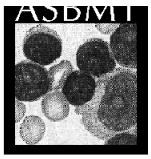

Mechanisms of Osteoporosis After Hematopoietic Cell Transplantation

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ABSTRACT

Osteopenia and osteoporosis are common complications of bone marrow and peripheral blood stem cell transplantation. Bone loss occurs in 50% to 60% of patients treated with the most common preparatory regimens. The major causes of transplant-related bone loss are primary hypogonadism (low estrogen and testosterone), secondary hyperparathyroidism due to low serum calcium, and posttransplant steroid therapy. Other transplant-related treatments that induce bone loss are discussed. Trabecular bone is particularly vulnerable to transplant-related therapies. The spine and hip contain 50% to 75% trabecular bone and are most at risk for fracture after hematopoietic cell transplantation (HCT). The structure of bone and the bone cells that are involved in maintaining skeletal integrity are discussed, followed by a discussion of the transplant-related therapies that have been shown to cause damage to bone and lead to bone loss. Recommendations for patients undergoing HCT include (1) evaluation of bone mineral density either shortly before or shortly after transplantation and appropriate intervention and monitoring based on the results; and (2) evaluation of estrogen and testosterone levels after HCT and replacement when appropriate; and (3) administration of bisphosphonate therapy to all patients on steroids for >2 months. Early intervention and prevention of bone loss can have a tremendous clinical impact for patients undergoing HCT because once significant bone loss has occurred, it is difficult to reverse.

KEY WORDS

Bone marrow transplantation • Osteoporosis • Osteoclast

INTRODUCTION

Osteoporosis is a skeletal disorder characterized by low bone mass and disturbances of the microarchitecture of the bone tissue. This pathologic process results in enhanced bone fragility and consequent increase in fracture risk [1]. Osteoporosis or low bone mineral mass occurs in more than 50% of patients after hematopoietic cell transplantation (HCT) [2,3]. Bone loss and fracture manifest as pain and loss of function, and they have a negative impact on quality of life.

The pathogenesis of transplantation bone disease is multifactorial and incompletely understood. Uncoupling of bone formation and bone resorption is the primary cause of osteoporosis, resulting in overactive osteoclastic bone resorption or underactive bone osteoblastic formation. The most common cause of osteoporosis is attributed to estrogen and testosterone deficiency associated with menopause and aging. Factors that predispose patients to osteoporosis include a diet low in calcium, low vitamin D levels from insufficient sunlight, genetic factors, and endocrine or renal insufficiency. Hematopoietic cell transplantation and posttransplant therapies can affect bone homeostasis by several mechanisms: inducing premature menopause and/or hypogonadism, directly poisoning bone cells, and elevating parathyroid hormone (PTH) levels by affecting the absorption and handling of calcium and magnesium. This review will discuss mechanisms that regulate normal bone remodeling and the transplantation-related factors that cause bone mineral loss and osteoporosis. The effect on bone metabolism of these therapies will also be discussed, as will mechanisms of action of anti-osteoporosis therapies.

NORMAL BONE REMODELING

The skeleton serves 2 principal functions: it is a structural support for mechanical needs and a storage reservoir

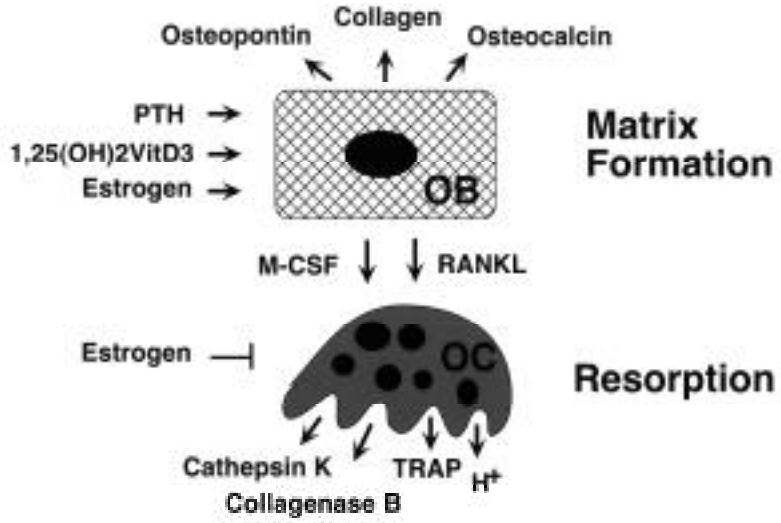
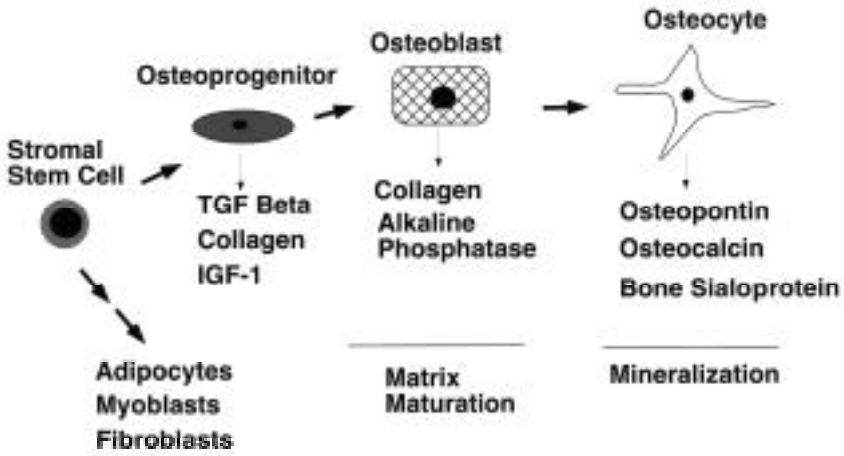


Figure 1. *Bone cell function. The bone remodeling unit consists of an osteoblast and an osteoclast. Osteoblasts (OB) produce bone matrix proteins and regulate the incorporation of calcium and phosphate in this organic matrix. OB also regulate osteoclasts (OC) through the secretion of macrophage colony-stimulating factor, RANK ligand, and other cytokines. Osteoclasts produce proteases and H^+ protons that degrade the calcified bone matrix. Parathyroid hormone–activated vitamin D ($1,25[OH]_2VitD3$) and estrogen regulate bone remodeling chiefly through their actions on osteoblasts [9].*

of calcium and phosphate for metabolic demands. Cortical bone forms the solid outer wall of all bones and accounts for 80% of bone mass [4]. Trabecular or cancelous bone, the honeycomb-like structure in contact with the bone marrow, represents 20% of the bone mass. The skeleton is constantly remodeled and regenerated by a dynamic process of resorption and replacement. The 2 principal cell types responsible for bone remodeling are the osteoclast, which resorbs bone, and the osteoblast, which forms bone. Osteoclasts and osteoblasts are organized in groups called basic multicellular units (BMU) [5] (Figure 1). The average life span of a BMU is 6 months, and it is estimated that there is complete renewal of the entire skeleton every 10 years [5].

CELLULAR COMPONENTS OF BONE

Osteoclastic activation is the initial step in the remodeling sequence. The osteoclast, a multinucleated hematopoietic cell derived from the monocyte/macrophage lineage, forms a ruffled membrane border and secretes a variety of specialized factors, including proteases, collagenases, phosphatases, and protons that break down the bone matrix, releasing mineral ions into the extracellular fluid. Osteoclastic differentiation and activation are regulated principally by osteoblasts. The osteoblast is a mesenchymally derived stromal cell that is involved in the formation of the organic bone matrix and the mineralization of new bone. The periosteum and bone marrow are important sources of mesenchymal osteoprogenitor cells that give rise to osteoblasts, chondrocytes, adipocytes,



Figur e 2. *Osteoblast lineage. The osteoblast is derived from a pluripotent mesenchymal stem cell. Osteoblasts secrete the organic bone matrix proteins. As osteoblasts mature, they secrete proteins important for the calcification of the bone matrix and develop into the terminally differentiated osteocyte. The osteocyte is embedded within the calcified bone matrix and communicates with other osteocytes and periosteal osteoblasts via cellular extensions [6].*

Table 1. *Osteoblast-Secreted Proteins*

Bone Matrix Proteins	Growth Factors
Collagen Type I	RANK ligand
Alkaline Phosphatase	Macrophage colony-stimulating factor
Osteocalcin	Osteoprotegerin
Osteopontin	Interleukin-1
Bone Sialoprotein	Interleukin-6
Matrix Gla Protein	Tumor necrosis factor- α
Thrombospondin	Transforming growth factor- β
Fibronectin	Insulin-like growth factor 1
Vitronectin	
Fibrillin	
Osteonectin	
Decorin	
Fibromodulin	
Biglycan	

Osteoblasts secrete a variety of proteins critical to the formation of the bone matrix. Osteoblasts also regulate osteoclast formation and activation through the production of cytokines and growth factors [9,6].

and myoblasts [6,7], as seen in Figure 2. The cuboidal-shaped osteoblasts synthesize the bone matrix, consisting of collagen type I, alkaline phosphatase, transforming growth factor β (TGF-β), and insulin-like growth factor 1 (IGF-1). After bone formation is completed, the osteoblasts become flat, and these quiescent osteoblasts are termed "lining cells" (Figure 2). Some of these osteoblasts will become buried in the mineralized bone and become osteocytes, with an extensive network of cell projections connecting them to other osteocytes and osteoblasts. As they mature into osteocytes, osteoblasts synthesize bone mineralization factors, including osteopontin, bone sialoprotein, and osteocalcin (Table 1) [6].

GROWTH FACTORS, CYTOKINES, AND HORMONES THAT INFLUENCE BONE DEVELOPMENT

The development and function of osteoclasts and osteoblasts are regulated by a variety of hormones, growth factors, and cytokines (Tables 2 and 3) that are susceptible to dysregulation following hematopoietic cell transplantation. The principal growth factors involved in bone remodeling are IGF-1, TGF-β, and bone morphogenic proteins (BMPs) (Table 2). IGF-1 and TGF-β are present in high concentrations within the bone matrix and stimulate osteoblastic replication and enhanced bone collagen and matrix synthesis by osteoblasts [8,6]. TGF-β inhibits osteoclast function and promotes osteoclast apoptosis, resulting in an overall decrease in bone resorption [5,9]. Therefore, the release of TGF-β stored in the bone matrix during bone resorption acts to inhibit further resorption and to induce bone formation. Transplant-related therapies interfere with these protective regulatory mechanisms by stimulating excess bone resorption through the induction of an excess of osteoclastic activating factors, discussed below [10].

Cytokines are important for growth and differentiation of osteoclasts (Table 3). The cytokines that stimulate osteoclasts are made primarily by osteoblasts in response to PTH and parathyroid hormone–activated vitamin D [1,25$(OH)_2$VitD3] (Figure 1, Table 1). Transplantation-induced hypogonadism and immunosuppressive therapy can trigger the production of these osteoblast-derived cytokines, resulting in inappropriate osteoclast activation. The most important cytokines regulating osteoclast differentiation and function are the newly identified RANK ligand (also called TRANCE, OPGL) [11,12] and macrophage colony-stimulating factor (M-CSF) [13-15]. RANK ligand, a tumor necrosis factor (TNF) receptor family member, is an osteoclast-activating factor that is critical to osteoclast development and function. Osteoblast-derived RANK ligand binds the RANK receptor on immature and mature osteoclasts, causing their differentiation and activation [12,16,17]. Osteoprotegerin (OPG) is expressed by a variety of tissues, including lung, bone, kidney, and liver, and it inhibits osteoclastic activation by binding RANKL and preventing RANKL binding to RANK [18]. OPG has been shown to prevent osteoporosis and increase bone mineral density when administered to rodents [18,19]. The mechanism through which RANK signaling affects osteoclasts is incompletely understood; however, RANKL/OPG/RANK regulation of osteoclasts is under intense investigation and will likely play an important role in study of transplantation-related bone loss. M-CSF has been shown to be critical to osteoclast and macrophage survival during maturation [20,21]. Other cytokines important for growth of osteoclasts are listed in Table 3. Interleukin (IL)-6 has been implicated in bone loss associated with estrogen withdrawal in the mouse, as well as the osteoclastic activation observed in myeloma patients [9,22].

PTH and 1,25$(OH)_2$VitD3 are the principal regulators of calcium homeostatis for most terrestrial vertebrates, including humans [23,24] (Figure 3). PTH increases circulating calcium levels by several mechanisms. It stimulates the differentiation of committed osteoclast progenitors to fuse and form mature multinucleated osteoclasts, and it also activates preformed mature osteoclasts to resorb bone, thereby releasing calcium into the serum [23]. The activation of osteoclasts by PTH occurs indirectly via stimulation of osteoblasts. PTH binds receptors on osteoblasts, resulting in the production of the following critical osteoclast maturation

Table 2. *Osteoblast Lineage Regulation*

Growth factors	
Insulin-like growth factor 1	Stimulatory
Transforming growth factor- β	Stimulatory
Bone morphogenic protein 2, 4, and 7	Stimulatory
Basic fibroblast growth factor	Stimulatory
Platelet-derived growth factor	Stimulatory
Systemic hormones	
Parathyroid hormone	Stimulatory
Parathyroid hormone–related protein	Stimulatory
1,25$(OH)_2$VitD3	Stimulatory

Bone formation and remodeling are regulated by growth factors and systemic hormones. Bone morphogenic protein, insulin-like growth factor 1, and transforming growth factor-β are the principal growth factors that regulate osteoblast formation, differentiation, and matrix formation. Parathyroid hormone and 1,25$(OH)_2$VitD3 induce osteoblasts to secrete cytokines and growth factors that will induce osteoclast proliferation and activation [6].

Table 3. *Osteoclast Lineage Regulation*

Systemic Hormones	
Calcitonin	Inhibitory
Growth factors	
Transforming growth factor- β	Inhibitory
Epidermal growth factor	Stimulatory
Platelet-derived growth factor	Stimulatory
Transforming growth factor- α	Stimulatory
Cytokines	
RANK ligand	Stimulatory
Osteoprotegerin	Inhibitory
Macrophage colony-stimulating factor	Stimulatory
Interleukin-6	Stimulatory
Interleukin-1	Stimulatory
Tumor necrosis factor	Stimulatory
Interferon- γ	Inhibitory

Osteoclast maturation, proliferation, and activation are regulated primarily by cytokines secreted from osteoblasts. RANK ligand and macrophage colony-stimulating factor are the 2 primary regulatory factors involved in maturation and are necessary for mature cell function [12]. Transforming growth factor-β is secreted by osteoblasts, stored in high levels in the bone matrix, and is released during bone resorption and inhibits further osteoclast proliferation [9]. Calcitonin is secreted from the medullary cells of the medulla and inhibits osteoclast activation [9].

and activation factors: RANKL, M-CSF, IL-6, IL-1, and TNF (Table 1) [23]. Transplant therapy–related toxicity of the kidney and gut could result in calcium losses, which would induce PTH secretion and osteoclast bone resorption and bone loss. Glucocorticoids decrease serum calcium levels and induce the secretion of PTH, resulting in increased bone resorption (by mechanisms discussed below) [25].

Vitamin D is biologically inert and must undergo 2 successive hydroxylation reactions in the liver and the kidney to become the biologically active 1,25-dihydroxyvitamin D [1,25$(OH)_2$VitD3]. 1,25$(OH)_2$VitD3 increases bioavailable calcium by increasing the intestinal absorption of calcium and phosphate. Like PTH, 1,25$(OH)_2$VitD3 acts indirectly to stimulate resorption by stimulating osteoblasts to produce RANKL and M-CSF, as described above. 1,25$(OH)_2$VitD3 also regulates important osteoblastic bone matrix genes like osteocalcin, osteopontin, and alkaline phosphatase by binding the specific vitamin D DNA-responsive elements present within the promoter regions of these genes [24]. Vitamin D is made in the skin by the action of sunlight. Transplant patients spend prolonged periods out of the sun and often consume a vitamin D–deficient diet. Vitamin D is rare in foods: the major nutritional sources are fatty fish and fortified dairy products [24]. In addition, problems with liver and kidney dysfunction in these patients can further block formation and activation of 1,25$(OH)_2$VitD3.

MECHANISM OF TRANSPLANTATION-RELATED OSTEOPOROSIS

Transplantation-associated osteoporosis is a side effect of heart transplantation [26], liver transplantation [27], lung transplantation [28], and bone marrow transplantation [2,3,29]. Immunosuppressive therapy, including cyclosporine (CSP), FK506, and glucocorticoids, is one of the principal factors leading to bone loss and osteoporosis [10]. Hypogonadism, kidney dysfunction, and malabsorption of calcium also contribute to bone loss (Figures 3 and 4). Total body irradiation and cranial irradiation can decrease growth hormone (GH) levels, which leads to a decrease in the IGF-1/insulin-like growth factor binding protein (IGFBP)-3 ratio. The GH/IGF-1 system induces the proliferation of the epiphyseal growth plate until sex hormone–mediated epiphyseal closure occurs [30-32]. Thus, total body irradiation (TBI) and cranial irradiation have important negative effects on skeletal growth in children and adolescents. Hematopoietic cell transplantation can induce bone loss and osteoporosis because of direct toxic effects of radiation therapy, chemotherapy, and cytokine therapy on bone cells and gonadal and pituitary hormone secretion [10].

HYPOGONADISM

Estrogen plays a vital role in maintaining bone mass. During menopause, women undergo significant bone loss [33]. Also, testosterone plays an important role in the maintenance of the male skeleton; however, it appears that testosterone's skeletal effects may in part be mediated by aromatization of testosterone to estradiol [34]. Ovarian insufficiency develops in 63% to 96% of premenopausal women who receive adjuvant chemotherapy for breast cancer [35]. Women older than 40 years are particularly at risk of chemotherapy-induced menopause [35]. Ovarian insufficiency occurs in up to 92% to 100% of women after TBI and high-dose chemotherapy. With chemotherapy as the sole conditioning regimen, ovarian failure occurs in up to 70% of patients [3]. Impaired spermatogenesis occurs in up to 75% of HCT patients; however, testosterone levels are often normal [36,37]. TBI also decreases growth hormone secretion, which can lead to decreased IGF-1 production and possibly hypogonadism [2,30]. Testicular and adrenal androgen levels are important in maintenance of bone mass in men [38], but estrogen also plays an important role in male bone mass, particularly in the growing skeleton [38,39]. Thus, both loss of estrogen in women or decreased levels of androgens in men can occur posttransplantation and can contribute to bone loss.

Estrogen receptors are expressed in both osteoblasts and osteoclasts [40]. Estrogen suppresses osteoclast formation and activity; estrogen deficiency leads to overactivity of osteoclasts compared with new bone formation [33]. Osteocyte apoptosis is increased in estrogen-deficient women [41]. Estrogen increases osteoblast transcription of IGF-1 and TGF-β and represses osteoblast transcription of osteoclast-activating factors (Figure 4). Therefore, estrogen increases bone formation and suppresses bone resorption [40]. Estrogen deficiency induces osteoblast production of osteoclast-activating factors (RANKL, IL-6) and suppresses bone formation [9]. Induction of osteoblast secretion of IL-6 in patients in an estrogen-deficient state is likely an important mechanism of bone loss in hypogonadism [22]. Although mechanisms whereby sex steroid deficiency mediates bone loss are incompletely understood [42], it is critical to monitor gonadal function after HCT and to replace hormones when appropriate to prevent hypogonadal bone loss.

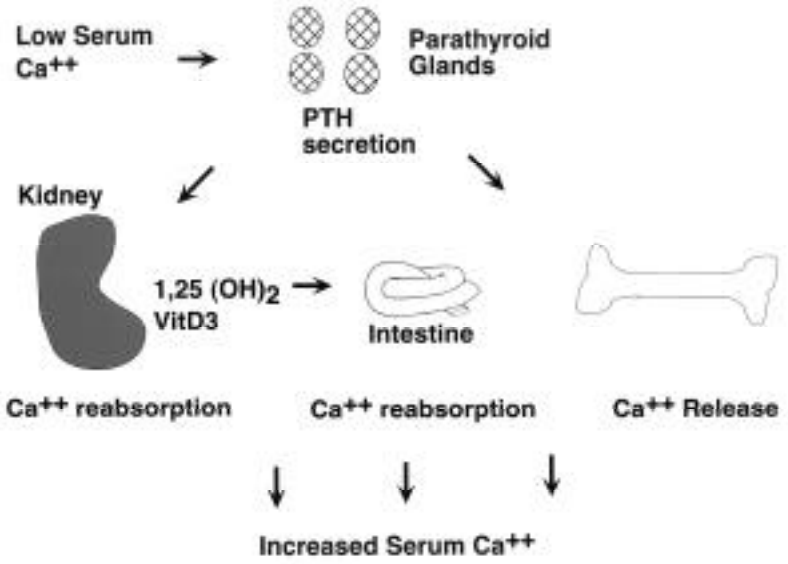


Figure 3. *Regulation of serum calcium levels. C cells in the parathyroid glands sense low serum calcium levels and secrete parathyroid hormone (PTH). PTH acts in bone to activate osteoclastic bone resorption. PTH also acts on the kidney to increase the production of active vitamin D (1,25[OH]$_2$VitD3) and to increase renal reabsorption of calcium renal tubule. 1,25(OH)$_2$ acts in the bone to activate osteoclastic bone resorption. 1,25(OH)$_2$VitD3 acts on the intestine to increase reabsorption of calcium in the gut. 1,25(OH)$_2$VitD3 and high serum calcium levels also downregulate PTH production in the parathyroid gland.*

GLUCOCORTICOID-MEDIATED BONE LOSS

Of patients taking steroids at doses greater than 7.5 mg per day for longer than 6 months, 30% to 50% will have an osteoporosis-related fracture [25]. Glucocorticoids have a greater effect on trabecular bone than on cortical bone; therefore, bone loss is most rapid and fractures are most likely in vertebrae, ribs, and the ends of long bones. Hematopoietic cell transplantation patients may have had prior glucocorticoid therapy as part of pretransplant chemotherapy, but it is unlikely that these short courses of glucocorticoids contribute to clinically significant bone loss [43]. Young people who have a high rate of bone turnover are very susceptible to glucocorticoid-mediated bone loss. Deceleration of growth and reduced total body calcium have been reported in children treated with oral and inhaled glucocorticoids [25].

The 2 key pathogenic mechanisms of glucocorticoid-induced osteoporosis are promotion of apoptosis of osteoblasts and osteocytes and inhibition of osteoblastogenesis [41]. Bone biopsies from patients who have received chronic steroids show a marked increase in osteoblast and osteocyte apoptosis [41]. Furthermore, the accumulation of apoptotic osteocytes may contribute to osteonecrosis. Abundant apoptotic osteocytes were found in femoral heads from patients with glucocorticoid-induced avascular necrosis, but not in patients with sickle cell disease or alcohol disease [41]. Glucocorticoids also decrease the expression of TGF-β type 1 receptor and antagonize the effects of BMP2 and IGF-1 [41]. Glucocorticoids induce expression of peroxisome proliferator activated receptor (PPAR)-γ2, which could account for increased adipogenesis in the bone marrow while osteoblast differentiation is suppressed [41]. Finally, the glucocorticoid receptor regulates many important osteoblast bone matrix proteins (such as collagen I, osteopontin, and alkaline phosphatase) [25] (Figure 4, Table 1). Thus, supraphysiologic glucocorticoids decrease bone formation by (1) inducing osteocyte and osteoblast apoptosis, (2) inhibiting osteoblast bone matrix synthesis, and (3) decreasing proliferation and differentiation of periosteal precursor cells [41].

Glucocorticoid therapy is a potent activator of osteoclasts. Glucocorticoids inhibit calcium absorption from the gut and increase calcium excretion in the kidney, resulting in low serum calcium levels. Low serum calcium is a strong stimulus for PTH production. Thus, glucocorticoids increase bone resorption indirectly by increasing PTH secretion [9,25]. Although glucocorticoids suppress follicle-stimulating hormone, lutenizing hormone, and prostaglandin E2, these effects do not appear to be clinically relevant [41,44]. Importantly, glucocorticoid-induced osteoporosis and bone loss can be prevented or decreased if recognized early and treated with bisphosphonates [41].

CYCLOSPORINE FOR GRAFT-VERSUS-HOST DISEASE

There is experimental evidence that CSP and FK506 contribute to bone loss associated with transplantation [10]. However, CSP is usually prescribed with glucocorticoids; consequently, it has been difficult to define its contribution to bone pathophysiology in humans. The exact mechanisms of CSP's effects on osteoblasts and osteoclasts are still unclear. Rats given CSP and FK506 (without glucocorticoids) have developed severe trabecular bone loss [45]. Bone turnover is accelerated, with evidence of increased resorption and formation. In the rat, the effect of cyclosporin A (CsA) on bone is independent of decreased kidney function [45]. Bone loss is largely prevented by anti-resorptive agents (like the bisphosphonates) in this model. In addition, CsA decreases osteoblast proliferation in vitro and decreases body stores of magnesium [10]. Mg is required for

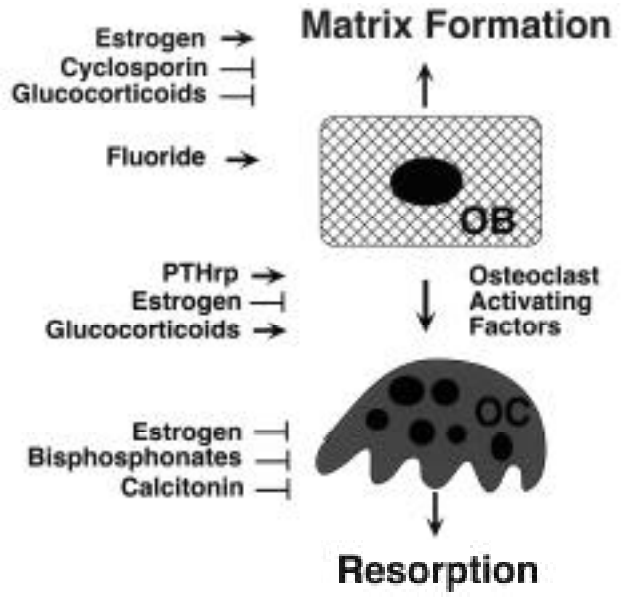


Figure 4. *Drugs on bone function. Bone remodeling can be affected by systemic hormones and transplant-related therapies. Cyclosporine, glucocorticoids, and estrogen deficiency decrease bone matrix formation. Glucocorticoids, tumor-derived parathyroid hormone–related protein, and estrogen deficiency increase osteoclast formation and activity through induction of osteoblast-derived cytokines. Osteoclast resorption and bone losses can be inhibited by bisphosphonates, estrogen, and calcitonin therapy [10].*

vitamin D hydroxylation and $1,25(OH)_2$VitD3 production; thus CsA can decrease $1,25(OH)_2$VitD3 levels [10].

RADIATION THERAPY AND CHEMOTHERAPY

Radiation therapy to bone has been associated with osteoporosis, increased bone fragility, radiation necrosis, radiation osteitis, increased fracture rate [46-48], and radiation-induced sarcoma of bone [49]. Total body irradiation is associated with hypogonadism, which could result in osteoporosis by the mechanisms outlined above. Irradiation can directly damage bone cells and has been shown to enlarge resorption lacunae and to increase osteoclast number and activity without an increase in bone formation, leading to increased bone resorption and bone porosity [50,51]. Bone irradiation is also associated with a decrease in viable osteocytes [52]. Radiation-induced effects on osteoclast and osteocyte populations improve with time [53].

Children treated for acute lymphoblastic leukemia (ALL) are particularly at risk of osteoporosis and reduced bone density [54]. Gilsanz et al. demonstrated that most of the reductions in bone mineral density occurred in the ALL patients who received cranial and spinal irradiation, and that chemotherapy for the disease plays only a minor role in bone loss after ALL [55]. On the other hand, Warner et al. found a correlation between prior methotrexate, ifosfamide, and bleomycin and reduction of spine bone mineral density, and between an exposure to 6-mercaptopurine and cisplatin and reduction of hip bone mineral density [54].

Patients undergoing standard-dose chemotherapy for cancer treatment can be at risk for treatment-related bone loss and osteoporosis. Analysis of the data indicates that the bone loss is not likely caused by the chemotherapeutic agents themselves but by a decrease in gonadal function induced by the chemotherapy [43,56]. Hypogonadism is a known cause of bone loss and osteoporosis, as described above. There are, however, some chemotherapeutic agents (methotrexate, ifosfamide, and cisplatin) that have a particular toxic effect on bone cell function, the mechanisms of which will be briefly described. Mechanisms of methotrexate (MTX) effects on bone have been studied because of the observation of bone loss in patients receiving MTX for rheumatoid arthritis. MTX induces osteopenia, probably because of a direct inhibitory effect on osteoblast proliferation and activity, resulting in a subsequent decrease in bone formation. MTX also stimulates osteoclast recruitment [40]. Patients undergoing high-dose ifosfamide treatment are at high risk of developing tubular nephrotoxicity and hypophosphatemia. Long-lasting hypophosphatemia may be associated with decreased osteoblastic activity and bone diseases, including rickets [57]. Cisplatin induces hypomagnesemia (resulting in decreased $1,25[OH]_2$VitD3 levels) and hypocalcemia (resulting in increased PTH levels). The net effect of cisplatin would be to induce bone resorption and exacerbate bone loss [58].

OTHER THERAPIES THAT INDUCE BONE LOSS

Heparin

One third of patients on long-term heparin therapy have reductions in bone density [59]. Heparin leads to trabecular bone loss, not only by increasing osteoclastic bone resorption but also by decreasing osteoblastic bone formation [59]. The biologic mechanisms through which heparin affects osteoblasts and osteoclasts are unknown. Low molecular weight heparin (LMWH) produces less bone loss and osteoporosis than unfractionated heparin [60]. Both heparin and LMWH decrease osteoblast function; however, heparin increases osteoclast number and activity and LMWH does not [59,60]. Heparin is sequestered in bone for an extended period, and its effects on bone loss are not immediately reversible when heparin therapy is stopped [59].

Thyroid Hormone

Many patients can become hypothyroid after HCT-conditioning regimens [37,61-64] and require thyroid hormone replacement. Thyroid hormone replacement closely monitored by a physician has not been associated with clinically evident bone loss; however, elevated levels of thyroid hormone (and thyroid hormone overreplacement) are associated with osteoporosis [65]. Thyroid hormone increases bone remodeling and directly stimulates osteoblast production of bone matrix proteins; however, it also increases osteoclast number, the number of resorption sites, and the ratio of resorptive to formative surfaces. The net result of elevated thyroid hormone levels is that osteoclast activity predominates, with a resultant loss of bone mass [66].

Granulocyte Colony-Stimulating Factor

G-CSF administration decreases bone mineral density in humans [15,67,68]. G-CSF is secreted by osteoblasts and stimulates osteoclastogenesis in vivo and in vitro but at a much lower rate compared with M-CSF and GM-CSF

[69]. G-CSF administration not only mobilizes granulocytes but also increases osteoclast numbers. G-CSF hematopoietic cell mobilization and G-CSF support after high-dose chemotherapy are often part of the standard HCT regimen. Short-term G-CSF induces osteoclastic bone resorption [68] and may be a factor in the pathogenesis of osteoporosis following HCT.

Kidney Dysfunction

Hematopoietic cell transplantation and its related therapies (cyclosporine, amphotericin, furosemide, and other diuretics) can result in renal dysfunction. This dysfunction could lead to decreased $1,25(OH)_2$VitD3 production and magnesium and calcium wasting, which would result in elevated levels of PTH and resultant osteoclastic bone resorption, possible bone formation problems, and overall bone losses. Renal bone disease (renal osteodystrophy) can be caused by acidosis, hyperphosphatemia, low serum $1,25(OH)_2$VitD3, and secondary hyperparathyroidism [70].

Malabsorption

Gastrointestinal losses of calcium and magnesium resulting from graft-versus-host disease, chemotherapy and radiation therapy, and infectious and antibiotic-associated diarrhea could play a role in transplantation-related osteoporosis by stimulating PTH secretion in response to low serum calcium and vitamin D [23] (Figure 3).

Osteoclast Engraftment and Stem Cell Reinfusion

High-dose chemotherapy with stem cell rescue has been associated with an increase in markers of bone resorption and a decrease in markers of bone formation [3]. Increase in cytokines, G-CSF, and TNF secretion could cause osteoclastic activation [71], and damage of resident osteoblasts by myeloablative therapies may contribute to diminished osteoblast function [52,53].

Tumor-Mediated Osteoporosis

Myeloma, certain types of lymphoma, and breast cancer have a propensity for spread and growth in the bone through the formation of osteolytic bone lesions. Many of these tumors produce osteoclast-activating factors, which enlist normal host osteoclasts to resorb bone [72]. Once the calcified and organic bone matrix is destroyed, the tumor cells can expand in its place. Many of these patients have had marked overall bone losses induced by their disease prior to stem cell transplantation and consequently are at high risk for clinically significant bone loss with the preparative regimen and after HCT regimens. The osteoclast-activating factors produced by myeloma, breast cancer, and some lymphomas include PTHrp, IL-6, RANKL, IL-1, and other uncharacterized factors [9].

THERAPIES FOR TRANSPLANTATION-RELATED OSTEOPOROSIS

Diagnosing hypogonadism (low estrogen or testosterone) after hematopoietic cell transplantation and instituting hormone replacement where feasible is the most important intervention to prevent bone loss. There are cases, however, where hormone replacement is not indicated and the extent of bone loss is so great that other interventions are necessary. Calcium, vitamin D repletion, and bisphosphonate therapy are mainstays of treatment. For example, the most effective therapy for glucocorticoid-induced bone loss is the early administration of bisphosphonates [41]. Bisphosphonate suppression of bone resorption has been known for more than 30 years. Bisphosphonates are analogues of pyrophosphate, and modifications of the 2 lateral chains on the carbon atom affect the potency of the compound [73]. Bisphosphonates bind to the bone mineral and are then taken up by osteoclasts during their initiation of resorption. In vitro and in vivo studies also show that bisphosphonates inhibit osteoclast activity, induce osteoclast apoptosis, and decrease osteoclast recruitment by acting on osteoblasts [74]. The exact molecular mechanism is incompletely understood. Fisher et al. have recently shown that nitrogen-containing bisphosphonates like alendronate and pamidronate act directly on osteoclasts to inhibit the rate-limiting step in the cholesterol biosynthesis pathway essential for osteoclast function [75]. Inside the osteoclast, alendronate interferes with the generation of geranylgeranyldiphosphate by inhibition of an enzyme in the cholesterol biosynthesis pathway, resulting most likely in a loss of prenylation of GTP-binding proteins that control cytoskeletal function, vesicular trafficking, and apoptosis. Many bisphosphonates are currently available that differ in their potency to inhibit osteoclastic resorption. For example, pamidronate is 100 times more potent than etidronate, and alendronate is 1000 times more potent than etidronate.

Other treatment and preventive therapies for osteopenia/osteoporosis include: (1) use of the lowest dose of glucocorticoid with the shortest half life, (2) weight-bearing exercise, (3) calcium intake of at least 1500 mg per day, and (4) maintenance of serum $1,25(OH)_2$VitD3 at upper limits of normal levels. Mundy et al. have recently demonstrated that the statins (drugs that inhibit 3-hydroxy-3-methylglutaryl coenzyme A [HMG CoA] reductase) increase bone formation in rodents by increasing expression of BMP2 [76]. Statins such as Lovostatin are frequently used to lower cholesterol, and their role in preventing osteoporosis and increasing bone mineral density is currently under investigation.

CONCLUSION

Hematopoietic cell transplantation and its related therapies can result in clinically significant bone loss. Induction of hypogonadism, alteration of serum calcium levels, induction of PTH, and direct toxic effects on the bone remodeling unit (osteoclasts and osteoblasts) result in net bone resorption and loss of bone mass. Early recognition of this problem and early intervention can decrease the negative impact of these therapies on bone mineral density, fracture risk, and overall quality of life.

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